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Small Dense Ldl: A Potent Biomarker For Cardiovascular Diseases

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Abstract:
This study aims to review and appraise regarding the available evidence related to the establishment of Small dense LDL as potent biomarker for the diagnosis of cardiovascular diseases. It also enlightens about the LDL subfraction and its association with the risk of cardiovascular diseases. Differences in low density lipoprotein (LDL) composition and size have been linked to coronary heart disease (CHD) risk and its progression. The clinical relevance has been well documented in various trials since the 1950s. Many recent clinical trials strongly reinforce the relevance of LDL heterogeneity measurement and the impact on CVD risk prediction and outcomes. The determination of LDL heterogeneity improves CVD risk prediction and helps in guiding appropriate treatment.
Key words*: sdLDL, cardiovascular diseases, LDL subfractions, atherosclerosis*

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I INTRODUCTION:

High incidence of atherosclerosis and associated cardiovascular diseases (CVD) urges to elaborate the causes and the risk factors responsible for disease progression. Elevated LDL cholesterol level is a significant marker for coronary heart disease [1]. However, therapies with statins and other lipid lowering drugs are efficient in lowering these levels and also help in prevention of thrombosis [2, 3]. Evidences from the studies have shown development of CHD in individuals despite of normal LDL cholesterol levels. Studies in recent years have shifted focus on LDL sub fractions rather than LDL itself. LDL particles are heterogeneous and have subfractions of different size, density and composition. Small dense LDL is highly dense form of LDL and has shown great extent of atherogenic effect. High atherogenic potential of SDLDL is due to its high susceptibility to oxidation as well as low affinity to bind with hepatic LDL receptor. Despite of all the medications given, reduction in low density lipoprotein cholesterol (LDL-C), a pool of cardiovascular disease remains[4]. Therefore, the focus shifts on two key points Firstly, that many individuals develop cardiac disorders despite of low levels of LDL cholesterol and secondly several studies disclose that due to heterogeneity of LDL molecule, its subparticles may have more discriminatory potential in development of CHD. Numerous clinical studies have been conducted to establish the link between the composition of circulating LDL particles and the risk of atherosclerosis and CVD development. According to the current consensus, 2 main phenotypes, A and B, are defined based on plasma LDL profile, with intermediate A/B phenotype lying in between. The phenotype A shows dominance of large buoyant LDL (lbLDL) and the phenotype B is characterized by the presence of sdLDL. Many researchers have emphasized the presence of Phenotype B in a number of metabolic disorders like obesity and type 2 diabetes and is considered as a risk factor of coronary heart disease (CHD) [5]. Moreover, it is also reported that this phenotype has strong association with the elevated levels of plasma triglyceride (TG) level, reduced HDL cholesterol (HDL-C), and high-hepatic lipase activity [6] .The predominance of sdLDL is currently accepted as a risk factor for CVD by the National Cholesterol Education Program (NCEPIII). LDL particles are known to show high variation not only in density and size, but they may vary in chemical composition. This variation is caused due to the series of changes they undergo while in blood circulation [7]. Among these particles, lipoprotein(a) which contains an additional lipoprotein molecule bound to apolipoprotein B, has been characterized as an additional cardiovascular risk factor. [8]Special focus is now on detection and measurement of modified LDL particles as these types of LDL can be a better marker for increased CHD, atherosclerosis, although their levels in blood might be low when compared with native LDL.

II LDL SUBFRACTIONS

LDL is broadly defined as lipoprotein fraction with density ranging from 1.006 to 1.063 g/ml, which can be isolated by various laboratory methods. This also includes the intermediate density lipoprotein (IDL) and very lowdensity lipoprotein (VLDL). More precisely, LDL is known to have a density from 1.019 to 1.063 g/ml. Ultracentrifugation and gradient gel electrophoresis (GGE) with their modifications are widely used for LDL analysis. In most of the studies using these methods, LDL particles are classified into 3 or 4 subclasses, including large (LDL I), intermediate (LDL II), small (LDL III), and, in some studies, very small (LDL IV) LDLs [9]. LDL III and LDL IV (when discerned) are referred to as sdLDL. However, the classification of LDL based on different analytical methods lacks uniformity, and care should be taken while comparing the results of clinical studies employing different methods. Small, dense low-density lipoproteins (sdLDL) have been increasingly studied as a better marker for cardiovascular disease outcomes. They were initially described by Krauss to be associated with relative increases in plasma triglyceride and apolipoprotein B and posited to potentially underlie a familial predisposition to CHD [10]. Austin has produced a large body of research further linking triglycerides and sdLDL [] as well as posting sdLDL as a risk factor for CHD, albeit based only on case control and cross-sectional studies. Two phenotypes of LDL were identified pattern A with LDL > 25.5 nm (large and buoyant) and pattern B with LDL ≤ 25.5 nm (small and dense)[11] .

There are several factors which suggest that sdLDLs are highly atherogenic. These include:

- They display higher penetration of the arterial wall.
- They have lower binding affinity for the LDL receptor.
- They have prolonged half life compared to the larger more buoyant LDL.
- They have a reduced resistance to oxidative stress compared to LDL.
- The surface lipid layer of sdLDL has a reduced content of free cholesterol and an increased content of free cholesterol contribute to enhanced oxidative susceptibility.
- Studies have shown a 2 to 3 fold increase in risk of CHD among individuals with pattern B phenotype .
- sdLDL has also been shown associated with both coronary and non coronary forms of atherosclerosis and is a risk factor for peripheral arterial disease [5]

III METABOLISM OF SDLDL – C

Berneis et.al. reported about the origin of sdLDL . They elucidated two pathways for its formation. Both the theories involved triglycerides availability. Hypertriglyceridemia that results from either increased production or decreased catabolism of Triglycerides directly influences LDL and HDL composition and metabolism. For example, the hypertriglyceridemia of insulin resistance is a consequence of adipocyte lipolysis that results in free fatty acid (FFA) flux to the liver and increased VLDL secretion. Higher VLDL Triglyceride output activates cholesteryl ester transfer protein, which results in triglyceride enrichment of LDL and HDL. The triglyceride content within these particles is hydrolyzed by hepatic lipase, which results in small dense LDL and HDL paricles [12]. This theory for the formation of small dense LDL is supported by many researches. Due to the sequence of reactions and alterations SDLDL particles have less conent of phospholipids, cholesterol and cholesterol esters but the amount of TG in their composition remains same.[13, 14]

However, some theories have also supported a different pathway for sdLDL. It was originally thought that sdLDL was formed in the circulation simply by delipidation of lbLDL [15]. However, there is now convincing evidence that sdLDL is formed directly from precursor lipoproteins secreted by the liver.The byproduct of triglycerides may lead to foam cell formation in a manner analogous to modified LDL. In addition triglycerides also share composition with classic LDL particles. Their composition includes presence of apo B and Cholestryl esters. They also possess particles which lead to progression of atherosclerosis. Occurrence of small Dense LDL is mostly accompanied by hypertriglyceridemia, reduced HDL cholesterol levels, abdominal obesity and many other series of alterations [16]. These alterations lead to increased susceptibility to thrombosis. Rise in plasma TG leads to accumulation of VLDL. This is the condition when small dense LDL formation starts. VLDL undergoes lysis and causes generation of LDL particles in the density range that have prolonged existing time of approximately 5 days compared to native LDL or intermediate density lipoproteins. The presence of this species triggers the formation of small dense LDL. Their structural conformation does not allow them to bind to the LDL receptors. This contributes to the fact that their retention period in blood is longer [17].

IV METHODS OF IDENTIFICATION

According to previous studies, LDL subfractions were separated by ultracentrifugation. This method allows to seprate the LDL subparticles on the basis of their floating rate. Another method used for separating LDL particles is gradient gel electrophoresis. This method is utilized for separating LDL subparticles according to their size and density.

Ultracentrifugation method have shown that 3 subclasses exists for LDL i.e. LDL I, II,III. Few studies also reported the presence of another class LDL IV. LDL I & II belong to the phenotype A, while LDL III, IV belongs to phenotype B which is considered to be atherogenic [18].

Besides, Ultracentrifugation method, Gradient gel Electrophoresis (GGE) and NMR are also widely used for analysis of subfractions of LDL. This method is purely based on electrophoretic mobility of LDL particles. The electrophoretic mobility depends on the shape and size of lipoprotein particles. Studies using GGE separation have shown four distinguished subclasses of LDL : LDL I (large LDL, peak diameter 26.0–28.5 nm), LDL II (intermediate LDL, 25.5–26.4 nm), LDL III A and B (small LDL, 24.2–25.5 nm), and LDL IV A and B (very small LDL, 22.0–24.1 nm). Many studies show that there is strong correlation between size and density of LDL particles analysed by both methods i.e. ultracentrifugation as well as Gradient gel Electrophoresis [19].

Many studies employed nuclear magnetic resonance technique to analyse the LDL subclasses but the results were not associated with the results of other two techniques. There was significant difference in the results which were achieved using Gradient Gel Electrophoresis. NMR is a method that basically involves a library of reference spectra. These spectra of lipoprotein subclasses are incorporated into a computer program. A new automated enzymatic assay was also developed to analyze small dense LDL and whether sdLDL is a risk factor for coronary heart disease. Another follow up study conducted in Japanese community utilized homogenous assay for direct measurement of sdLDL levels [20].

Some laboratories have used indirect methods also to analyse LDL subfractions. This includes estimation by ratios of routinely done parameters eg. Fasting Triglycerides, HDL-C, TG/HDL-C, LDL-C/ Apolipoprotein-B. Among all these methods, fasting Triglycerides was found to be having statistically significant correlation with small dense LDL. This also helps to establish a strong connection between small dense LDL levels and cardiovascular diseases. Numerous Studies done in coronary heart disease patients showed the expression of SDLDL-C in 22.7 % subjects having fasting Triglycerides levels <150mg/dl but in subjects having fasting TG of 70 mg/dl only 4.2 % showed the expression of small dense LDL. The group having fasting TG >250 , 100% subjects showed the presence of small dense LDL. This helps to establish the fact that small dense LDL might be an independent marker for cardiovascular diseases irrespective of TG levels. In few other studies they have analyzed the levels by using some equations which use the values of routinely done lipid parameters [21,22].

Various other methods have also been used in studies to analyze LDL fractions like High Performance liquid chromatography, dynamic light scattering and also ion mobility analysis [23, 24].

V SDLDL and risk of cardiovascular disease

TABLE no.1 : shows the evidences of previous studies establishing strong relation between SDLDL and cardiovascular disease risk.

STUDY	**YEAR**	**FINDINGS**
ARIC –study [25]	2014	There was strong association of small dense LDL with future CHD events even in individuals who were at low CVD risk based on their LDL-C level
Obese- Thai population study [26]	2015	Increased prevalence of sdLDL in Thai obese population which was similar to the findings observed in European & American population.
Study on older men with stable CAD [27]	2018	sdLDL was proved to be more effective secondary prevention marker as compared to LDL for predicting future cardiovascular events.
Prospective study in Japanese community [28]	2020	sdLDL cholesterol was associated with the development of CHD despite of normal or low LDL levels.
Copenhagen Heart study [29]	2020	High levels of sdLDL indicated high risk of MI and Atherosclerotic cardiovascular disease subjects
Prospective Framingham offspring study [30]	2021	sdLDL contributes significantly to atherosclerotic cardiovascular disease and is most atherogenic parameter

VI Statin Therapy and Treatment response for SdLDL

Elevated sdLDL levels have also been reported in patients who are on statin therapy. Study conducted on patients treated with rouvastatin and with average LDL levels of 54mg/dl, sdLDL was seen associated with Coronary heart disease [31]. Diet modification, exercise, restricting calories can help in reducing small dense LDL. Study also concluded that supplementation of Fish oil also reduces small dense LDL [32, 33].

VI CONCLUSION

Small Dense LDL has strong association with cardiovascular diseases and is also linked to Atherosclerotic cardiovascular disease events. The findings of studies and researches already done on small dense LDL are accordant with the evidences that suggest that sdLDL has greater atherogenic potential than any other lipid parameter. Effect of statin therapy is also seen to lower LDL and triglyceride levels but has no affect on small dense LDL levels. Thus, we can conclude that measuring sdLDL levels helps in assessment of cardiovascular disease risk and it is a potent marker for predicting coronary heart diseases. Future investigations should be made on therapies targeting the small dense LDL levels.

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